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Uptake of dietary micronutrients from artificial diets by larval *Heliothis virescens*

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Abstract

Micronutrient assimilation from artificial diet by larvae of *Heliothis virescens* during selenium (Se) supplementation was studied. The metal content of pupae and plugs of the artificial diet on which they had developed from hatching was analyzed by inductively coupled plasma-mass spectrometry. Levels of the metals Cr, Co, Fe, Mg, Mn, Ni, Se, Na, and Zn were not bioaccumulated from the diet regardless of the amount of Se added to the diet. Only pupal Cu and Mo bioaccumulation were found to be altered significantly by dietary Se supplementation. Larvae fed Zn, which was found in higher levels in pupae than diet, had a deleterious response to increasing levels of dietary Zn. Larvae fed Cr, found in higher levels in diet than in pupae, were not adversely affected when increasing levels of Cr were added to the diet. Based on this analysis, metals were identified that might well impact the fitness of a given colony of insects in relation to their diet.

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1. Introduction

Micronutrients are metals or metal-containing inorganic compounds utilized as enzymatic cofactors or as intracellular messengers. Dietary micronutrient intake can affect the growth, development and immunocompetence of vertebrates (Wellinghausen et al., 1997; Beck et al., 2004). The essentiality of these elements in insect nutrition is well established (Dadd, 1985); however, the optimal micronutrient requirements of insects are largely unknown. We recently demonstrated that dietary supplementation with selenium (Se) (in the form of selenite) boosts the immunocompetence of the larval lepidopterans, the cabbage looper (*Trichoplusia ni*) (Popham et al., 2005), and the tobacco budworm (*Heliothis virescens*) (Shelby and Popham, 2006), elevating larval resistance to *per os* challenge with a fatal baculovirus infection. While the mechanism of Se-dependent baculoviral resistance is as yet unknown, the observation opens several new lines of investigation: (i) the

optimal dietary concentration range and formulation of Se; (ii) dietary Se assimilation, metabolism and excretion; (iii) Se-dependent biochemical or physiological processes in larval or adult insects; (iv) micronutrients required for optimal, or maximal fitness; and (v) interactions of other nutrients with dietary Se.

An immunostimulatory effect was observed when diet was supplemented with 5–25 parts per million (ppm) Se (Popham et al., 2005; Shelby and Popham, 2006). Higher levels of Se appeared to exceed the tolerable upper level, with slowed or disrupted larval development. Thus, for the species studied we reported an upper and lower boundary for Se concentration between beneficial supplementation and toxicity. These effects on larval immunocompetence indicate that the artificial diets commonly used to rear insects in the laboratory, which are sufficient to support growth, development and reproduction (measures of fitness), may have specific micronutrient deficiencies which could affect other types of bioassays. For example, interactions with the highly complex mix of phytochemicals present in foliar tissues, and largely absent from artificial diets, may alter the response of feeding larvae to Bt toxins

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(Tabashnik et al., 2003). Understanding of the influence of a single inorganic element on insect biology is clearly far more complex than a simple issue of dietary requirements.

Because of the importance of other metal micronutrients besides Se to vertebrate immunity (Wellinghausen et al., 1997; Wellinghausen and Rink, 1998; Erickson et al., 2000), and the stimulatory action of Zn on *Manduca sexta* hemocytes (Willot and Tran, 2002), and of Fe on *Galleria mellonella* immunity (Dunphy et al., 2002), we have evaluated the hypothesis that the dietary levels of additional micronutrients may impact the fitness or immunocompetence of insects reared on artificial diets. Assimilation and bioaccumulation of metals directly from the diet into tissues of the insect was studied.

In vertebrates, absorption and utilization of iodine, Zn, Fe, Cr, and vitamin A are supported by adequate dietary Se (Lyons et al., 2004). The observed immunomodulatory effects of Se supplementation could be caused by an antagonistic interaction with other vital micronutrients such as Zn, Cr, etc., or by a positive interaction with dietary toxins such as Hg (Jensen et al., 2006). Therefore, we investigated the hypothesis that dietary Se supplementation may affect the uptake or assimilation of other micronutrients from artificial diets commonly used to rear insects in the laboratory.

2. Materials and methods

2.1. Insects

H. virescens eggs were received from the North Carolina State University Dept. of Entomology Insectary. Their insectary colony was established from field insects collected in July of 2002. Larvae were reared individually on an artificial wheat germ-based diet (Catalog # F9781B, BioServe, Frenchtown, NJ) containing Wesson's Salt Mixture minerals with no added Se (Popham et al., 2005; Shelby and Popham, 2006). Larvae were reared under a photoperiod of 14 h:10 h (L:D) at 55% relative humidity at 28 °C.

2.2. Supplemented diets

Se-supplement diets were prepared with Se added to the stock culture medium in the form of Na_2SeO_3 (Sigma Chemical, St. Louis, MO, USA) at 5, 10, 25, and 50 ppm. Cr-supplement diets were prepared with added Cr in the form of $Cr(histidine)_3$ or $Cr(picolinate)_3$ at 0.5, 3, and 10 ppm. Zn-supplement diets were poured with Zn added in the form of $ZnSO_4$ or $ZnCl_2$ (Sigma Chemical, St. Louis, MO, USA) at 20, 40, 60, 80, 100, and 200 ppm. Diet without added dietary Se, Cr, or Zn was considered to be basal Se, Cr, and Zn, respectively. Larvae were monitored daily for death, pupation, and emergence. The presence of developmental irregularities including larval/pupal or pupal/adult intermediates was recorded during each study.

2.3. ICP-MS analysis of metal content

Individual pupae or small core samples of diet were placed in pre-tared vials, weighed, and oven dried at 65 °C for two or more days until no further loss of water mass was noted. Dry mass was calculated from these final dried samples. Pupae were chosen for analysis because larvae completely void midgut contents prior to pupation, minimizing contributions of diet contents within the digestive system. Three male and three female pupae from larvae reared on each different level of Se were analyzed in each group. Metal determinations were performed by the University of Missouri Research Reactor Analytical Services by inductively coupled plasma-mass spectrometry (ICP-MS) (Beauchemin, 2004). Samples were subjected to microwave digestion in nitric acid. The microwave digestion system was a CEM brand MDS-2000 with Advanced Composite Vessels (CEM Corporation; Matthews, NC, USA). During the microwave program, the setpoint pressure for the vessels was stepped up from 40 psi (hold 5 min), to 60 psi (hold 5 min), to 80 psi (hold 5 min), and to 100 psi (hold 10 min). Digestates (approximately 12 ml) were analyzed undiluted and with gravimetric dilutions ($200 \times$ and $1000 \times$ for most samples). Na, Mg, Mn, Fe, and Zn were quantitated from dilutions. All others were quantitated from digestates. The elements scandium and yttrium were included as internal standards in all digestates, dilutions and calibration standards. The ICP-MS used for all of the elements was a VG Elemental Axiom High-Resolution ICP-MS (Thermo Electron Corp., VG Elemental, Waltham, MA, USA). Metal concentrations were expressed as μg/g dry mass (ppm). The sum of all determined isotopes of each metal are presented. Two independent replicates were performed. Statistical comparisons were made with the Holm–Sidak pairwise multiple comparison method when a significant ANOVA value was found ($p < 0.05$) (SigmaStat, Systat Software Inc., Point Richmond, CA, USA).

2.4. Plasma in vitro virucidal activity assay

The *Helicoverpa zea* cell line, *Hz*AM-1, was maintained as monolayers at 28 °C in Excel 401 medium (JRH Biosciences, Lenexa, KS, USA) supplemented with 10% fetal bovine serum (Intergen Co., Purchase, NY, USA). Virucidal activity in larval *H. virescens* plasma was quantified by endpoint dilution assay as detailed (Popham et al., 2004). In brief, plasma dilutions were combined with *Helicoverpa zea* single nucleopolyhedrovirus (*Hz*SNPV) at a ratio of 3:1 (v/v) and allowed to incubate at 20 °C for 1 h. PBS was used as a control in the absence of plasma. Viral titers of these incubations were determined by endpoint dilution assay. *Hz*AM-1 cells were seeded at 5×10^4 cells/ml in P96 well plates (BD Falcon) and allowed to attach for 1 h. The cells were infected with dilutions of virus/plasma or virus/PBS at dilutions of 10^{-1}–10^{-6} and plates were incubated for 1 week at 28 °C. The plate wells were then

scored positive, if polyhedra were visible within two or more cells, or negative for viral infection, and the results were used to calculate the viral titer as the tissue culture infectious dose per ml ($TCID_{50}$/ml) of inoculum. Wild type HzSNPV isolate was used and amplified in HzAM-1 cells for the virucidal assay (Popham et al., 2004). Statistical comparisons were done using the Student–Newman–Keuls pairwise multiple comparison (SigmaStat, Systat Software Inc., Point Richmond, CA, USA).

3. Results

The amount of Se in diet is shown in comparison to the amount of Se found in pupae (Fig. 1). In basal diet, the amount of Se was determined to be 0.26 ± 0.01 ppm Se which was less than that found in male (0.66 ± 0.08 ppm Se) and female (0.68 ± 0.09 ppm Se) pupae fed basal diet throughout the larval stage. However, as dietary Se increased, the amount of Se available in the diet far exceeded the amount sequestered in pupae (Fig. 1). The amount of Se sequestered in male versus female pupae was not significantly different as the amount of Se increased (one-way ANOVA, $p = 0.630$).

Assimilation of eight metals did not change significantly as the concentration of Se was increased in the basal diet. Six of these metals were found to be more abundant in the diet than in pupae; Co, Cr, Fe, Mn, Na, and Ni (Table 1). Two metals, Mg and Zn, were found at higher levels in the diet than in pupae. However, Mg was significantly lower in the diet in replicate one but higher in the diet in replicate two. Only Na was found to be at significantly different levels in male versus female pupae with male pupae significantly higher than female pupae (ANOVA, $p < 0.001$).

Cu and Mo were present in basal diet at levels lower than what was present in pupae, but the amounts of both metals changed significantly as the concentration of Se was
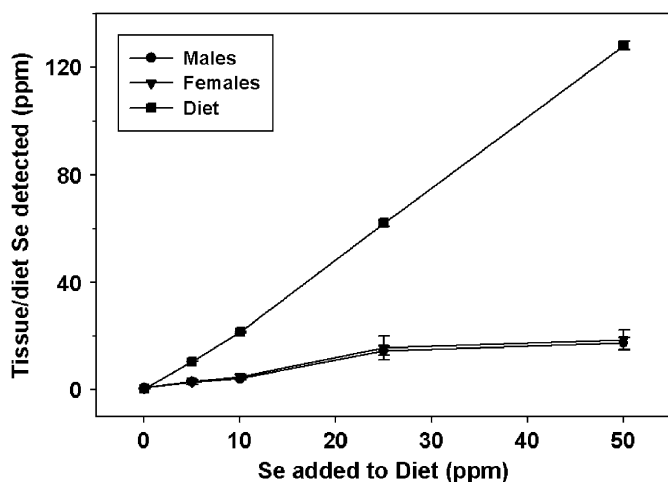

Fig. 1. As Se was added to the diet, the amount of available Se far exceeded the amount of Se sequestered in pupae [Mean $\pm$ SEM, $n = 3$ (males and females) or $n = 9$ (diet)].

Table 1
Metals measured by ICP-MS were present in basal diet either at levels below what was present in pupae or at levels above what was present in pupae

Metal	Males (ppm)[a]	Females (ppm)[a]	Diet (ppm)[b]
Metals present in diet at levels above what was present in pupae			
Co[59]			
Rep 1	0.0060 ± 0.0006	0.0058 ± 0.0006	0.0216 ± 0.0007
Rep 2	0.0078 ± 0.0006	0.0075 ± 0.0005	0.0193 ± 0.0005
Cr[52]			
Rep 1	0.0028 ± 0.0002	0.0042 ± 0.0004	0.3362 ± 0.0231
Rep 2	0.0229 ± 0.0113	0.0242 ± 0.0129	0.4400 ± 0.0138
Fe[56]			
Rep 1	34.5 ± 2.1	36.3 ± 2.1	257.9 ± 2.5
Rep 2	38.0 ± 2.5	35.2 ± 2.5	294.0 ± 3.0
Mn[55]			
Rep 1	34.0 ± 1.7	37.0 ± 1.7	45.9 ± 2.0
Rep 2	40.8 ± 2.1	38.6 ± 2.1	61.1 ± 2.6
Na[23]			
Rep 1	774.5 ± 38.0	320.2 ± 38.0	3828.7 ± 46.3
Rep 2	437.7 ± 31.7	156.5 ± 31.7	3826.5 ± 38.9
Ni[60]			
Rep 1	0.0509 ± 0.0087	0.0404 ± 0.0078	0.3100 ± 0.0087
Rep 2	0.0851 ± 0.0128	0.0888 ± 0.0144	0.4000 ± 0.0149
Metals present in diet at levels below what was present in pupae			
Mg[24&26]			
Rep 1	5294.1 ± 182.4	5329.8 ± 182.4	3888.6 ± 223.4
Rep 2	4486.7 ± 73.5	4435.1 ± 73.5	4888.1 ± 90.0
Zn[64&66]			
Rep 1	264.7 ± 7.9	283.3 ± 7.9	102.8 ± 9.6
Rep 2	285.0 ± 7.9	281.6 ± 7.9	104.6 ± 9.7

[a]Mean $\pm$ SEM, $n = 15$.
[b]Mean $\pm$ SEM, $n = 10$.

increased (Fig. 2). Cu changed significantly in both pupae and diet levels individually and between one another as Se was added to the diet (two-way ANOVA, $p < 0.001$). The amount of Cu in pupae remained above what was present in diet. Mb significantly decreased in pupae as Se was elevated (two-way ANOVA, $p < 0.001$) though there was no significant difference between male and female pupal Mo levels. The amount of Mo in diet did not change significantly as the amount of Se increased (one-way ANOVA, $p = 0.355$).

Zn was chosen from the group where the metal was in higher quantities in pupae than diet. $ZnSO_4$ was added to the diet in levels up to 200 ppm and the rate of pupation, emergence, and death measured (Fig. 3). A delay in emergence was noted at 20 ppm, the lowest addition of Zn (Fig. 3A). By 40 ppm Zn, there was a delay in pupation and emergence and an increase in death and the number of larvae that failed to successfully pupate increased (termed larval/pupal intermediates) (Fig. 3B). Over 80% of the deaths at 60 ppm were due to this failure to pupate. Beyond 60 ppm, as the amount of added Zn increased, more larvae died prior to pupation. By 200 ppm, 80% of the deaths
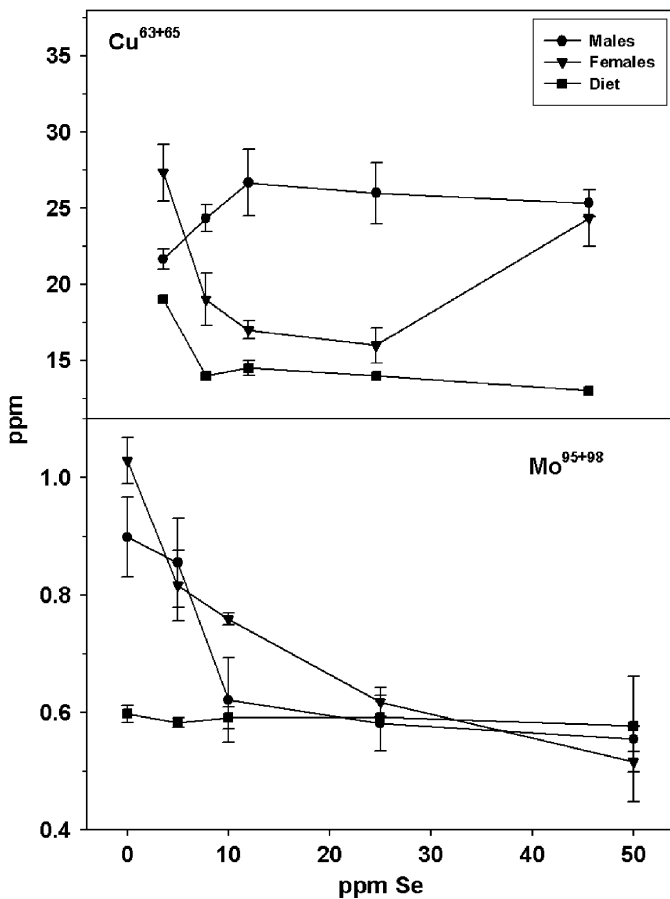
Fig. 2. The amount of Cu and Mb sequestered in pupae significantly changed as larvae were fed increasing levels of Se [Mean±SEM, $n = 3$ (males and females) or $n = 2$ (diet)].

Fig. 3. Elevated levels of dietary Zn sulfate resulted in an increase in larval mortality, delayed pupation, and delayed adult emergence of H. virescens compared to those reared on basal diet. (A) Rate of pupation and emergence; (B) final percent mortality, aborted pupation, pupation and emergence. Basal diet contained 103 ppm Zn. Diets were supplemented with 20–200 ppm Zn. Larvae were placed on diets as neonates ($n = 50$).

were in the larval stage. $ZnCl_2$ was also added to diet but was also found to be extremely toxic to larvae even at low levels (data not shown).

Cr, a metal higher in diet than pupae, was added to diet in the form of Cr(histidine)$_3$ at levels up to 10 ppm. No obvious differences were seen in pupation, emergence or death as the amount of Cr was elevated (Fig. 4A and B). Cr(picolinate)$_3$ was also added to diet as another form of exogenous Cr but exerted no different influence than added Cr(histidine)$_3$ (data not shown).

The effect of metals upon the immunocompetence of Cr and Zn supplemented H. virescens larvae was assessed by monitoring plasma virucidal activity against the Helicoverpa zea SNPV baculovirus using an endpoint dilution assay (Popham et al., 2004). Supplementation with from 30 to 1000 parts per billion (ppb) Cr(his)$_3$ or Cr(picolinate)$_3$ did not significantly alter plasma virucidal activity (data not shown). Supplementation with $ZnSO_4$ also showed no significant effect on plasma virucidal activity in the 20–40 ppm range; however significant inhibition was evident at 60 ppm Zn (Table 2). The latter concentration of Zn was far above the toxic range causing significant developmental delays, malformations, and mortality (Fig. 3B).
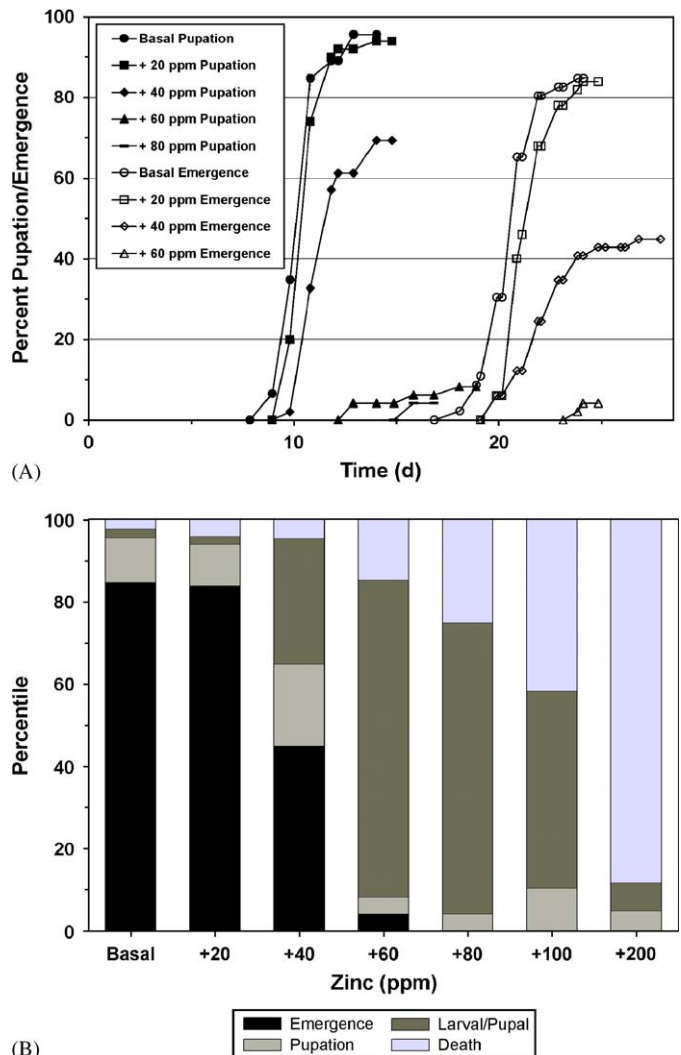
4. Discussion

The majority of studies using insects have focused upon the toxic effects of environmental pollution with heavy metals (Trumble et al., 1998; Vickerman et al., 2002a, b; Jensen and Trumble, 2003; Vickerman and Trumble, 2003; Hepburn et al., 2003; Vickerman et al., 2004). However several species of plants have evolved metal hyperaccumulation strategies, assimilation of toxic concentrations of the metals Cd, Co, Cr, Cu, Mn, Pb, Se, and Zn, which are thought to discourage herbivory (Hanson et al., 2003, 2004; Boyd, 2004). Although in general the metals content of herbivorous insects reflect the foliage upon which they have fed, elimination of high or toxic levels of selected metals such as Zn may occur (Pihlajamaki et al., 1989). We propose that subtoxic concentrations of metals present in hyperaccumulator and other plants, as well as in artificial
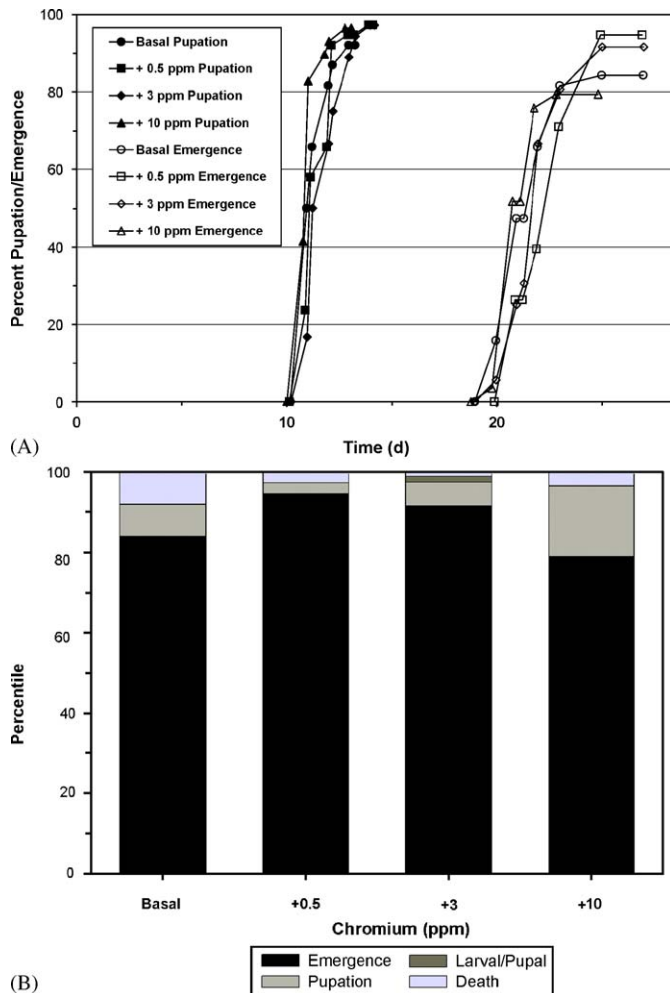
Fig. 4. Elevated levels of dietary Cr(histidine)$_3$ did not increase mortality or delay pupation and adult emergence times of *H. virescens* larvae compared to those reared on basal diet. (A) Rate of pupation and emergence; (B) final percent mortality, aborted pupation, pupation and emergence. Basal diet contained 0.39 ppm Cr. Diets were supplemented with 500, 3000, and 10,000 ppb Cr. Larvae were placed on diets as neonates ($n = 35$).

Table 2
Elevated levels of dietary zinc sulfate inhibited the virucidal activity in 5th instar larval *H. virescens* plasma

Treatment	Viral titer	SEM
PBS	4.01×10^6	3.08×10^5 a
Basal diet	8.64×10^4	3.26×10^4 b
+20 ppm Zn Diet	1.38×10^5	3.65×10^4 b
+40	1.55×10^5	3.78×10^4 b
+60	2.37×10^6	6.81×10^5 a

Basal diet averaged 103 ppm Zn. Letters indicate significant differences between treatments; $n = 4$, mean $\pm$ SEM.

diets, may impact insect immunocompetence in the field and in the laboratory. Wide scale intentional elevation of important micronutrients in grains and other foods by agronomic or by biotechnological means (biofortification) has been proposed in order to alleviate worldwide dietary deficiencies of Cu, Fe, I, Se and Zn (White and Broadley, 2005; Genc et al., 2005). Ironically, biofortification of crops with some micronutrients may also benefit some pest insects, while deterring others.

Our data support the hypothesis that dietary Se intake can influence the assimilation of other micronutrients. We observed that increasing concentrations of dietary Se decreased assimilation of Cu by female pupae, but increased Cu assimilation by male pupae. Developing males may require Se in order to assimilate Cu into male-specific tissues, such as testes for future sperm production (McFarlane, 1976). In *Drosophila melanogaster* three transporters actively accumulate Cu from the midgut lumen (Zhou et al., 2003). Cu is a cofactor in a number of important enzymes, including tyrosinase and phenoloxidase; and a deficiency leads to disruption of cuticular melanization (Zhou et al., 2003). Inclusion of a Cu chelator in *H. virescens* larval diet reduced survival following challenge with AcMNPV (Washburn et al., 1996), perhaps by reducing the activity of plasma phenoloxidase (Popham et al., 2004). Increased dietary Se reduced assimilation of Mo by female and male larvae, as seen in their pupal tissues. Dietary Se did not appear to influence assimilation of other elements. At present we have no compelling hypothesis that would explain these observations. We also do not know whether the influence of dietary Se on larval resistance to baculovirus challenge is a direct effect of Se, Se incorporated into selenoproteins, or results from altered assimilation of other metals.

Cr supplementation with Cr(picolinate)$_3$ or Cr(his)$_3$ has proved beneficial to vertebrates (Anderson et al., 2004). The more soluble supplement Cr(his)$_3$ was not superior to Cr(picolinate)$_3$ in our experiments. The latter supplement was shown to cause sterility and lethal mutations in *D. melanogaster* at concentrations above 260 ppm (Hepburn et al., 2003). However we observed no toxicity even at the highest levels of Cr(his)$_3$ supplementation. Our experiments demonstrate no net beneficial effect of Cr supplementation, and therefore the basal diet likely contains sufficient Cr for normal growth and physiology. However, definitive experiments will require a diet that can cause a *bone fide* deficiency of this micronutrient.

Zn is an important cofactor in many enzymes such as DNA, RNA polymerases, alkaline phosphatases, alcohol dehydrogenases (Wellinghausen et al., 1997). House crickets, *Acheta domesticus*, have dietary requirements for Cu and Zn (McFarlane, 1976). Male development was especially sensitive to deficiencies of these metals. In agreement with our data, he observed that both male and female development was affected by higher Zn concentrations. We observed a specific developmental lesion at the larval/pupal molt of *H. virescens* caused by excess Zn. The data indicate that levels of Zn in the basal diet are already approaching the upper end of the subtoxic range for this element. Definitive work on the Zn requirements of lepidopteran larvae will require the formulation of a low- or Zn-free artificial diet.

Turning to the practical implications of our findings, nutrients or phytochemicals in foliar tissues that may alter the immunocompetence of insect larvae include sterols (Mac Donald and Ritter, 1988), tannins, chlorogenic acids (Hoover et al., 1998; Hoover et al., 2000), Fe (Dunphy et al., 2002), Zn (Willot and Tran, 2002), and Se (Popham et al., 2005). Our results, and those of others, indicate that the immune responses of insects reared on artificial diets in the laboratory may differ substantially from insects fed a foliar diet or reared on plants. Specific nutritional deficiencies may alter the results of some bioassays using artificial diets; therefore caution in extrapolating laboratory-based work to the field is indicated. Moreover, mass-reared insects, intended for release as biological control agents, could conceivably suffer from immunosuppressive nutrient deficiencies or toxic accumulations of same, thus reducing their fitness and efficacy. In this paper, we have identified at least four metals that may alter immunocompetence against baculoviral infection in our artificial diet system.

Acknowledgments

References

Anderson, R.A., Polansky, M.M., Bryden, N.A., 2004. Stability and absorption of chromium and absorption of chromium histidinate complexes by humans. Biological Trace Element Research 101, 211–218.

Beauchemin, D., 2004. Inductively coupled plasma mass spectrometry. Analytical Chemistry 176, 3395–3415.

Beck, M.A., Handy, J., Levander, O.A., 2004. Host nutritional status: the neglected virulence factor. Trends in Microbiology 12, 417–423.

Boyd, R.S., 2004. Ecology of metal hyperaccumulation. New Phytologist 163, 563–567.

Dadd, R.H., 1985. Nutrition: organisms. In: Comprehensive Insect Physiology Biochemistry and Pharmacology, vol. 4. Pergamon Press, Oxford, pp. 313–390.

Dunphy, G.B., Niven, D.F., Chadwick, J.S., 2002. Iron contributes to the antibacterial functions of the haemolymph of *Galleria mellonella*. Journal of Insect Physiology 48, 903–914.

Erickson, K.L., Medina, E.A., Hubbard, N.E., 2000. Micronutrients and innate immunity. Journal of Infectious Disease 182 (Suppl 1), S5–S10.

Genc, Y., Humphries, J.M., Lyons, G.H., Graham, R.D., 2005. Exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations. Journal of Trace Elements in Medicine and Biology 18, 319–324.

Hanson, B., Garifullina, G.F., Lindblom, S.D., Wangeline, A., Ackley, A., Kramer, K., Norton, A.P., Lawrence, C.B., Pilon-Smits, E.A.H., 2003. Se accumulation protects *Brassica juncea* from invertebrate herbivory and fungal infection. New Phytologist 159, 461–469.

Hanson, B., Lindblom, S.D., Loeffler, M.L., Pilon-Smits, E.A.H., 2004. Se protects plants from phloem-feeding aphids due to both deterrence and toxicity. New Phytologist 162, 655–662.

Hepburn, D.D.D., Xiao, J., Bindom, S., Vincent, J.B., O'Donnell, J., 2003. Nutritional supplement chromium picolinate causes sterility and lethal mutations in *Drosophila melanogaster*. Proceedings of the National Academy of Sciences USA 100, 3766–3771.

Hoover, K., Alaniz, S.A., Yee, J.L., Rocke, D.M., Hammock, B.D., Duffey, S.S., 1998. Dietary protein and chlorogenic acid effect on baculoviral disease of noctuid larvae. Environmental Entomology 27, 1264–1272.

Hoover, K., Washburn, J.O., Volkman, L.E., 2000. Midgut-based resistance of *Heliothis virescens* to baculovirus infection mediated by phytochemicals in cotton. Journal of Insect Physiology 46, 999–1007.

Jensen, P., Trumble, J.T., 2003. Ecological consequences of bioavailability of metals and metalloids in insects. In: Bondari, K. (Ed.), Recent Research in Developments in Entomology, vol. 4. Research Signpost, Trivandrum, pp. 1–17.

Jensen, P.D., Johnson, L.R., Trumble, J.T., 2006. Individual and joint actions of selenate and methylmercury on the development and survival of insect detritivore *Megaselia scalaris* (Diptera: Phoridae). Archives of Environmental Contamination and Toxicology 50, 523–530.

Lyons, G.H., Stangoulis, J.C., Graham, R.D., 2004. Exploiting micronutrient interaction to optimize biofortification programs: the case for inclusion of selenium and iodine in the HarvestPlus program. Nutrition Reviews 62, 247–252.

Mac Donald, D.L., Ritter, K.S., 1988. Effect of host sterols on the sterol composition and virulence of a nuclear polyhedrosis virus of *Heliothis zea*. Lipids 23, 1107–1113.

McFarlane, J.E., 1976. Influence of dietary copper and zinc on growth and reproduction of the house cricket (Orthoptera: Gryllidae). Canadian Entomologist 108, 387–390.

Pihlajamaki, J., Vaananen, V.M., Koskinin, P., Nuorteva, P., 1989. Metal levels in *Laothoe populi* and Sphinx pinastri (Lepidoptera, Sphingidae) in Finland. Annales Entomologici Fennici 55, 17–21.

Popham, H.J.R., Shelby, K.S., Brandt, S.L., Coudron, T.A., 2004. Potent virucidal activity against HzSNPV in larval *Heliothis virescens* plasma. Journal of General Virology 85, 2225–2261.

Popham, H.J.R., Shelby, K.S., Popham, T.W., 2005. Effect of dietary selenium supplementation on resistance to baculovirus infection. Biological Control 32, 419–426.

Shelby, K.S., Popham, H.J.R., 2006. Dietary selenium supplementation elevates HzSNPV resistance of *Heliothis virescens* larvae. Journal of Invertebrate Pathology (in press).

Tabashnik, B.E., Carriere, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M., Zhao, J.-Z., 2003. Insect resistance to transgenic Bt crops: lessons from the laboratory and field. Journal of Economic Entomology 96, 1031–1038.

Trumble, J.T., Kund, G.S., White, K.K., 1998. Influence of form and quantity of selenium on the development and survival of an insect herbivore. Environmental Pollution 101, 175–182.

Vickerman, D.B., Trumble, J.T., 2003. Biotransfer of selenium: effects on an insect predator, *Podisus maculiventris*. Ecotoxicology 12, 497–504.

Vickerman, D.B., Shannon, M.C., Banuelos, G.S., Grieve, C.M., Trumble, J.T., 2002a. Evaluation of *Atriplex* lines for selenium accumulation, salt tolerance and suitability for a key agricultural insect pest. Environmental Pollution 120, 463–473.

Vickerman, D.B., Young, J.K., Trumble, J.T., 2002b. Effect of selenium-treated alfalfa on development, survival, feeding and oviposition

preferences of *Spodoptera exigua* (Lepidoptera: Noctuidae). Environmental Entomology 31, 953–959.

Vickerman, D.B., Trumble, J.T., George, G.N., Pickering, I.J., Nichol, H., 2004. Selenium biotransformations in an insect ecosystem: effects of insects on phytoremediation. Environmental Science and Technology 38, 3581–3586.

Washburn, J.O., Kirkpatrick, B.A., Volkman, L.E., 1996. Insect protection against viruses. Nature 383, 767.

Wellinghausen, N., Rink, L., 1998. The significance of zinc for leukocyte biology. Journal of Leukocyte Biology 64, 571–577.

Wellinghausen, N., Kirchner, H., Rink, L., 1997. The immunobiology of zinc. Immunology Today 18, 519–521.

White, P.J., Broadley, M.R., 2005. Biofortifying crops with essential mineral elements. Trends in Plant Science 10, 586–593.

Willot, E., Tran, H.Q., 2002. Zinc and *Manduca sexta* hemocyte functions. Journal of Insect Science 2, 6.

Zhou, H., Cadigan, K.M., Thiele, D.J., 2003. A copper-regulated transporter required for copper acquisition, pigmentation, and specific stages of development in *Drosophila melanogaster*. Journal of Biological Chemistry 278, 48210–48218.